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Human erythrocyte hemolysis assay

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An abbreviated version of this protocol was published in eLIFE in Feb 2022

Protein-lipid interaction at low pH induces oligomerization of the MakA cytotoxin from *Vibrio cholerae*

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Detailed protocol

1) Collect 1 mL of fresh blood from a healthy human donor.
2) Centrifuge the entire blood sample at 500 x g for 10 minutes immediately after collection.
3) Separate the blood plasma from the rest of the blood.
4) Suspend RBCs in PBS (5-10 mL), then centrifuge at 500 x g for 5 min.
5) Wash the RBC pellet in PBS twice by centrifuging at 500 x g for 5 min.
6) Discard the PBS and resuspend the RBCs in a sodium citrate buffer (120 mM) of the desired pH.
In our case, we used a 120 mM sodium citrate buffer with a pH of 5.0, 6.5, or 7.4.
Note: Adjust the pH using either 2.5M NaOH or 2.5M HCl.
7) Adjust the final concentration of RBCs in a sodium citrate buffer (120 mM) to 0.25 % at a pH of your choice.
In our case, we used a sodium citrate buffer (120 mM) with a pH of 5.0, 6.5, or 7.4.
Note: Adjusting the RBC concentration up to 1 % provides similar results.
8) Add MakA in varying concentrations and incubate the eppendorf tubes at 37 °C for 90 min or 5 h.
9) Lyse the RBCs with Triton X-100 (0.1 % final volume) as a positive control.
It is essential to employ a positive control for all pH-adjusted samples (i-e., use individual control for pH 5.0, 6.5, or 7.4).
10) At the end of the treatment, centrifuge the eppendorf tubes for 5 min at 500 x g.
11) Transfer 100 mL of the supernatant into each well of the 96-well plate.
12) Spectrophotometrically monitor the release of hemoglobin by measuring the absorbance at 545 nm to indicate red blood cell lysis.
Plot the data relative to the RBCs treated with Triton X-100 (0.1% final volume).

Chemicals:
tri-Sodium citrate, Analytical reagent grade, Batch# 0689824, Fischer Scientific.

Triton X-100, Laboratory grade, Lot # SLBM3870V, Sigma Aldrich.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Nadeem, A. and Nyunt Wai, S. (2022). Human erythrocyte hemolysis assay. Bio-protocol Preprint. bio-protocol.org/prep1903.
2. Nadeem, A., Berg, A., Pace, H., Alam, A., Toh, E., Ådén, J., Zlatkov, N., Myint, S. L., Persson, K., Gröbner, G., Sjöstedt, A., Bally, M., Barandun, J., Uhlin, B. E. and Wai, S. N.(2022). Protein-lipid interaction at low pH induces oligomerization of the MakA cytotoxin from *Vibrio cholerae*. eLIFE. DOI: 10.7554/eLife.73439